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Spontaneous Neoplasms in Fishes. VI. Thyroid Tumors in Marine Fishes

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(Plates I-IX)

Introduction

THYROID TUMORS in marine fishes are of special interest because these animals live in an environment normally rich in iodine. The following species have been previously reported with such growths: the Pacific dogfish, *Squalus suckli* (=*suckelyi*), by Cameron & Vincent (1915); two species of Mediterranean sea basses, *Serranus scriba* and *Serranus cabrilla*, by Marsh & Vonwiller (1916); a European sparoid, *Box vulgaris* (=*Boops boop*), by Johnstone (1924); the northwestern Atlantic barndoor skate, *Raia laevis* (=*stabuliformis*), by Burwash (1929); and the shark-sucker, *Echeneis naucrates*, by Schlumberger & Lucké (1948). The single specimen of *Box vulgaris* and the two of *Raia laevis* were taken by deep-sea trawl and appear to be the only marine fishes reported with naturally occurring thyroid enlargements. The other species, together with those reported here, developed thyroid tumors while in captivity.

Among the fishes exhibited in the New York Aquarium, the following marine species of teleosts (bony fishes) have been found with thyroid growths: the broad killifish or sheepshead minnow, *Cyprinodon variegatus* Lacépède; the blue angelfish, *Angelichthys isabelita* Jordan & Ritter; and the common killifish or mummichog, *Fundulus heteroclitus* (Linnaeus). These tumors have been previously reported (Nigrelli, 1940, 1943, 1952), but they were not described in detail. The present contribution deals with the histopathology of these growths.

Material and Methods

In May, 1939, it was discovered that 10 sheepshead minnows (4 males and 6 females) in a group of 30 fish had developed thyroid enlargements. The fish had been collected in the spring of 1938 at Sandy Hook Bay, New Jersey, and had become established as a reproducing population in a tank containing a number of green morays, *Gymnothorax funebris*, which had been in captivity from five to ten years. It was the death of several of these morays in December of 1938 that called attention to this tank. An analysis of the water showed that the specific gravity had increased from 1.026 to 1.042 and that the pH had shifted from 8.2 to 7.7, and these factors were recorded as contributory causes of the deaths. It was at this time that evidence of the thyroid tumors in the sheepshead minnows was first seen. Five months later, all of the minnows were removed from the tank, and those that were in a moribund condition were sacrificed for anatomical and histological studies.

The blue angelfish was a five-year-old female specimen which had been brought in with a collection of fishes from Key West, Florida. The branchial swelling in this fish was first noticed in August, 1940, and it was removed from the tank in a dying condition in December of the same year. The pH of the water was 8.3 and the specific gravity 1.023, both factors being within the normal range of sea water.

In September of 1950, a number of mummichogs were brought in from Long Island Sound at Mamaroneck, New York, and placed in a tank containing a Kemp's turtle. All of the fish, except one female, were eventually caught and eaten by the turtle, and in January of 1951 a branchial enlargement was noticed in this fish. It was removed to a smaller tank in the laboratory and kept under observation until May of that year when it was sacrificed. In June, 1951, another collection was made from Long

Island Sound in the vicinity of Orchard Beach (Bronx, New York) and the mummichogs were again placed in the tank with the turtle. Of the six fish which survived, four (three females and one male) developed growths which were visible externally in from one to six months. These fish were sacrificed on June 30, July 11, July 27 and November 26. An analysis of the water during this period showed that the pH varied from 7.1 to 7.4, the specific gravity from 1.017 to 1.018 and the iodine content was determined to be 17-20 gammas per liter.[1] These factors are well within the expected range for the brackish waters that killifish frequently inhabit.

It should be pointed out that the routine diet for marine fishes used at the New York Aquarium consists of fresh clams, shrimp and salt water fish—foods relatively rich in iodine.

The pathological tissues were prepared for microscopical examination after decalcification. Paraffin sections were cut at three and six microns and some were stained with Toludine blue, Giemsa's, Mallory's and Masson's methods; others were treated with either Delafield's or Harris' hematoxylin-eosin stain.

Observations

The fishes with thyroid tumors showed various degrees of emaciation probably caused, in part, by dysphagia. No exophthalmia was evident. Those that showed marked external swellings had labored respiration and were in anoxia, resulting from the following conditions: (1) impairment of the water-pumping mechanism because of destruction of cartilage, bone and muscle of the hyoid and branchial complexes by proliferated tumor tissues; (2) circulatory embarrassment, the result of pressure caused by the thyroid growth on the heart, ventral aorta and afferent and efferent vessels; and, (3) destruction of respiratory elements (gill lamellae) by invading thyroid tissue.

The thyroid tumors in the mummichogs were more or less alveolar and filled the entire hyoid and mandibular regions. In two of the fish the tumor grew into the pharyngeal and branchial cavities, and in one case (Plate I, Figure 1) extended beyond the external limits of the operculum. There was considerable infiltration into bones and muscles supporting these regions, and pressure effects were noted on the heart, blood vessels and esophagus (Plate I, Figure 2). The esophagus was reduced to a narrow slit which microscopically showed a papillary-like, thickened mucosa with much of the epithelium sloughed into the lumen (Plate II, Figure 3). This condition was undoubtedly responsible for the emaciated condition of the fish. The growths in the sheepshead minnows were more compact than those seen in the mummichogs, but there was infiltration into similar structures and extensive destruction of the lamellae of the first three pairs of gills (Plate V, Figure 10; Plate VI, Figure 11). The tumor in the blue angelfish was nodular and involved much of the anterior hyoid region. A number of the lamellae of the anterior parts of all the gills were affected (Plate VIII, Figure 15).

Microscopically, the growths in the three species were characterized by extensive proliferation of follicular and interfollicular elements. Although the tumors were not encapsulated, they were fairly regular at their free surfaces. The greatest proliferation occurred in regions of least impedance, usually into the loose, often edematous connective tissue supporting the hyoid, mandibular and branchial structures (Plate II, Figure 4). The destruction of muscle, cartilage and bone was direct; the proliferated tissue invaded and destroyed the perimysium, perichondrium and periosteum. The muscle fibers in more distant regions were frequently disoriented as the result of pressure by the tumor. The fibers in the immediate regions were invaded through interseptal pathways (Plate III, Figure 5) and were split into smaller units and hyalized (Plate VI, Figure 11). Cartilage and bone, even when infiltrated, often were not completely destroyed. There was always a certain amount of regeneration as indicated by the proliferation of chondroblastic and osteoblastic cells (Plate III, Figure 5; Plate VI, Figure 12). There was little or no inflammatory reaction present but there was some increase in vascular and connective tissue. In some instances, the *adventitia* of larger blood vessels was penetrated by the tumor tissue (Plate IV, Figure 7) but no metastases or tumor thrombi were found.

The tumors in all three species of fishes were more or less pathognomically similar, but the histological details differed in certain respects. The growths in the mummichog and in the sheepshead minnow were classified as interacinous adenomas. In both, the epithelium had reached considerable proportions, and many new alveoli lined by round, cuboidal or columnar cells were formed between larger (older?) acini (Plate II, Figure 4; Plate VI, Figure 12). The nuclei of these cells were normal in appearance, but no mitotic figures were seen. The cytoplasm had the characteristic polariza-

[1] The iodine determination was made by Dr. Viateur Rousseau, Department of Chemistry, College of Mount St. Vincent, New York City. This is part of the chemical and physical analyses of the goitrogenic sea water which will form the basis of future experimental studies.

tion of basal and apical zones, and, in the mummichog tumor, intracellular red or orange colored colloid droplets were demonstrated with Masson's stain (Plate V, Figure 9). The presence of intracellular colloid was indicative of a highly stimulated gland. The intrafollicular colloid was either basophilic or acidophilic in reaction and often vacuolated. In some follicles, round lymphoid cells were present, and in such instances the colloid was highly vacuolated and colored red and blue with Mallory's or Masson's stain (Plate IV, Figure 8).

In addition to the many small, newly formed alveoli, the tumor in the sheepshead minnow showed extensive areas consisting of afollicular elements (Plate VII, Figures 13 and 14). These were small, round or irregularly shaped cells with homogenous acidophilic cytoplasm and with nuclei that were smaller and more compact than the follicular cells. In these regions, the tumor had the appearance of a metaplastic growth. There was some indication (Plate VII, Figure 13) that these cells were derived from follicular epithelium.

The thyroid growth in the blue angelfish was sufficiently atypical in microscopic structure to be interpreted as a true neoplasm without question. The tumor was a large, nodular mass which appeared to be an adenocarcinoma. (Plate VIII, Figures 15 and 16; Plate IX, Figures 17 and 18). There were zones of small alveoli with and without lumina, cords of secondary alveoli and typical follicles in which the colloid, when present, showed a diminished staining reaction. In some areas, the growth had a papillary-like appearance. There was an increase in fibrous tissue which circumscribed various groups of alveoli (Plate VIII, Figure 16). The stroma was highly vascular, containing many sinusoids (Plate IX, Figure 18) and small, thick-walled blood vessels. The epithelial cells, which were cuboidal or columnar, often were binucleated, but no mitotic figures were found.

Discussion

It seems self-evident that factors other than iodine deficiency must be considered as the cause of thyroid tumors in marine fishes. In most marine teleosts, as in freshwater ones, the thyroid gland is a non-encapsulated organ, the number, size and distribution of the follicles showing both individual and species variation (Guternatsch, 1910, 1911, a, b; Lynn & Wachowski, 1951). It has been suggested that changes in the thyroid gland of fishes may result, either directly or indirectly, from changes in oxygen tension (Duerst, 1941), in light intensity (Rasquin, 1949), and in salinity (Olivereau, 1948). These reports indicate that the structure of the thyroid gland in fishes is easily altered by changes in environmental conditions. Other factors which should be considered as possibly inducing thyroid hyperplasia in marine fishes in aquaria are alterations in the calcium-magnesium ratio of the water and the increased activity forced on the captive fish by the presence of an aggressive tank-mate or potential predator. Experiments testing these possibilities are now in progress.

Schlumberger & Lucké (1948) reviewed the literature on tumors of fish thyroids and in their discussion suggested that the majority of cases reported probably represent simple hyperplasia and colloid storage. Nevertheless, in some instances, as in the thyroid tumor of the blue angelfish, there is ample evidence that these growths are true neoplasms.

The tumors in the mummichog and sheepshead minnow must also be considered malignant. In both species, the thyroid epithelium reached considerable proportions. The follicles were lined with columnar or cuboidal cells and many new acini, with and without lumina, had developed together with numerous afollicular epithelial cells. Ewing (1940) referred to a somewhat similar type of growth found in human goiters as an interacinous adenoma, and indicated that this condition may be a low-grade neoplastic process. It is apparent, however, that the factors which limit the growth of interacinous adenomas in human thyroids are not present in teleosts. In the latter, the tumors are comparatively fast growing and do not remain stationary after reaching certain dimensions. Instead, the growth process continues, infiltrating and destroying much of the surrounding structures. In bony fishes, therefore, interacinous adenomas are malignant growths and represent a true neoplastic process although there may be relatively little change in the histological character of the tissue. In this connection it might also be pointed out that Willis (1948) emphasized that even in human beings, where the thyroid is encapsulated, no clear separation of epithelial hyperplasia, benign and malignant thyroid tumors is possible.

Summary

Thyroid tumors were found in the following marine fish kept at the New York Aquarium: *Fundulus heteroclitus* (mummichog or common killifish), *Cyprinodon variegatus* (sheepshead minnow or broad killifish), and *Angelichthys isabelita* (blue angelfish).

The tumors in the mummichog and in the sheepshead minnow were characterized by a

considerable increase in follicular and afollicular elements and were, therefore, classified as interacinous adenomas. The tumor in the angelfish was identified as an adenocarcinoma, and consisted of many small, poorly formed acini with and without lumina in which the colloid appeared abnormal. An increase in connective tissue and vascular stroma was also present.

All the tumors were malignant, infiltrating and destroying gill tissue, cartilage, bone and muscle of the hyoid-mandibular-branchial complex. There was invasion into the *adventitia* of some of the large blood vessels but no metastases were found.

References

Burwash, Frances M.
1929. The Iodine Content of the Thyroid of Two Species of Elasmobranchs and One Species of Teleost. Contr. Canadian Biol. and Fish., N. S., 4: 117-120.

Cameron, A. T. & S. Vincent
1915. Notes on an Enlarged Thyroid Occurring in an Elasmobranch Fish (*Squalus sucklii*). J. Med. Res., 27: 251-256.

Duerst, J. U.
1941. Die Ursachen der Enstehung des Kropfes. Bern: Hans Huber, Publ. [Quoted by Schlumberger & Lucké, 1948].

Ewing, James
1940. Neoplastic Disease. W. B. Saunders Company. Philadelphia and London, xiii, 1160 pp.

Guternatsch, J. F.
1910. The Structure, Distribution and Variation of the Thyroid Gland in Fish. J. Amer. Med. Assoc., 54: 227.
1911a. The Thyroid Gland of the Teleosts. J. Morph., 21: 709-782.
1911b. The Relationship Between the Normal and Pathological Thyroid Gland of Fish. Bull. Johns Hopkins Hosp., 22: 152-155.

Johnstone, Jas.
1924. Disease Condition in Fishes. Rep. for 1923 on the Lancashire Sea-Fisheries Lab. No. No. XXXII: 99-129.

Lynn, W. Gardner & Henry E. Wachowski
1951. The Thyroid Gland and Its Functions in Cold-Blooded Vertebrates. Quart. Rev. Biol., 26: 123-168.

Marsh, M. C. & P. Vonwiller
1916. Thyroid Tumors in Sea Bass (*Serranus*). J. Cancer Res., 1: 183-186.

Nigrelli, Ross F.
1940. Mortality Statistics for Specimens in the New York Aquarium, 1939. Zoologica, 25: 525-552.
1943. Causes of Diseases and Death of Fishes in Captivity. Zoologica, 28: 203-216.
1952. Spontaneous Neoplasms in Fishes. VI. Thyroid Tumors in Marine Fishes. Cancer Res., 12: 286, Abstract.

Olivereau, Madeline
1948. Influence d'une diminution de salinité sur l'activité de la glande thyroïde de deux Téléostéens marins: *Muraena helena* L., *Labrus bergylta* Asc. Compt. Rend. Soc. Biol., 142: 176-177.

Rasquin, Priscilla
1949. The Influence of Light and Darkness on Thyroid and Pituitary Activity of the Characin *Astyanax mexicanus* and its Cave Derivatives. Bull. Amer. Mus. Nat. Hist., 93: 501-531.

Schlumberger, H. G. & Balduin, Lucké
1948. Tumors in Fishes, Amphibians and Reptiles. Cancer Res., 8: 657-754.

Willis, R. A.
1948. Pathology of Tumors. The C. V. Mosby Company, St. Louis. xiii, 992 pp.

EXPLANATION OF THE PLATES

Figures 1-9: interacinous adenoma of the thyroid of the mummichog or common killifish, *Fundulus heteroclitus.*

PLATE I

FIG. 1. Thyroid tumor almost filling the entire branchial region and extending beyond the external limits of the operculum, which has been cut away. Note the extensive destruction of gill tissue. About 2½ ×.

FIG. 2. Sagittal cut to show the extent of the growth in the pharyngeal and oral cavities. Pressure effects on the heart and esophagus are especially noticeable. About 3½ ×.

PLATE II

FIG. 3. Section at the level of the esophagus. Note the papillomatous appearance of this structure and desquamated epithelial elements in the lumen. H-E. 100 ×.

FIG. 4. Edge of the tumor growth. Numerous small acini, with and without colloid or lumina, are scattered between larger typical-appearing follicles. Note the pressure effect on the large blood vessel in the upper left hand corner. H-E. 100 ×.

PLATE III

FIG. 5. Details of the tumor tissue at the edge of the growth showing infiltration into muscle tissue. H-E. 450 ×.

FIG. 6. An area showing the effects of the growth on bone. Note the proliferation of osteoblastic cells. H-E. 450 ×.

PLATE IV

FIG. 7. Large vein in which the *adventitia* is invaded by thyroid tumor cells. H-E. 450 ×.

FIG. 8. Large follicle with acidophilic and basophilic, vacuolated colloid infiltrated by round lymphoid cells. Masson's. 950 ×.

PLATE V

FIG. 9. Follicular cells showing intracellular colloid droplets, indicating a highly stimulated gland. Masson's. 950 ×.

Figures 10-14, interacinous adenoma in the sheepshead minnow or broad killifish, *Cyprinodon variegatus.*

FIG. 10. Section of the thyroid tumor showing the extent of the growth. Mallory's. 25 ×.

PLATE VI

FIG. 11. Thyroid tumor showing follicles with acidophilic (black) or basophilic (gray) colloid. Note the effects on muscle tissue. Mallory's. 50 ×.

FIG. 12. An area of the tumor showing the invasion and partial destruction of bone. H-E. 100 ×.

PLATE VII

FIG. 13. Afollicular elements around a single follicle. H-E. 450 ×.

FIG. 14. Details of afollicular cells. The cytoplasm is acidophilic and the nucleus is smaller, more compact than in the cells of the follicles.

Figures 15-18, adenocarcinoma of the thyroid of the blue angelfish, *Angelichthys isabelita.*

PLATE VIII

FIG. 15. Massive nodular and highly vascular tumor in the anterior gill region. About 1½ ×.

FIG. 16. In some regions the growth exhibited a papillary-like arrangement. Note the compactness of the growth and the acini circumscribed by connective tissue. H-E. 50×.

PLATE IX

FIG. 17. Zone showing cords of secondary alveoli both with and without lumina. H-E. 450 ×.

FIG. 18. Details showing small acini in which the colloid presents a diminished staining reaction. The tumor is permeated with sinusoids. H-E. 950 ×.